Current Biology 24, 970–975, May 5, 2014 ©2014 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2014.03.015

Report

Germ-Granule Components Prevent Somatic Development in the *C. elegans* Germline

Dustin L. Updike,[1,2,*] Andrew Kekūpa'a Knutson,[1] Thea A. Egelhofer,[1] Anne C. Campbell,[2] and Susan Strome[1]
[1]Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA 95064, USA
[2]Kathryn W. Davis Center for Regenerative Biology and Medicine, Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672, USA

Summary

Specialized ribonucleoprotein organelles collectively known as germ granules are found in the germline cytoplasm from worms to humans [1]. In *Drosophila*, germ granules have been implicated in germline determination [2]. *C. elegans* germ granules, known as P granules, do not appear to be required for primordial germ cell (PGC) determination [3], but their components are still needed for fertility [4–6]. One potential role for P granules is to maintain germline fate and totipotency. This is suggested by the loss of P granules from germ cells that transform into somatic cell types, e.g., in germlines lacking MEX-3 and GLD-1 or upon neuronal induction by CHE-1 [7, 8]. However, it has not been established whether loss of P granules is the cause or effect of cell fate transformation. To test cause and effect, we severely compromised P granules by simultaneously knocking down factors that nucleate granule formation (PGL-1 and PGL-3) and promote their perinuclear localization (GLH-1 and GLH-4) [9] and investigated whether this causes germ cells to lose totipotency and initiate somatic reprogramming. We found that compromising P granules causes germ cells to express neuronal and muscle markers and send out neurite-like projections, suggesting that P granules maintain totipotency and germline identity by antagonizing somatic fate.

Results and Discussion

The germline cytoplasm of numerous animals contains unique "germ granules." Despite the discovery of germ granules decades ago, the role (or roles) of those granules and the fate of germ cells when germ granules are lost are not understood. In *Drosophila*, loss of germ granules prevents formation of primordial germ cells (PGCs) in embryos [10]. In *C. elegans*, failure to segregate P granules to the germline blastomeres (P cells) does not impair determination and early development of the germline [3]. Two studies have reported a correlation between transformation of germ cells toward somatic cells and the loss of P granules, suggesting that P granules may function to maintain totipotency in the germline. Upon neuronal induction by the transcription factor CHE-1, P granules are no longer detected in transformed germ cells [8]. Similarly, P granules are lost from germ cells that transform into somatic cell types in *mex-3 gld-1* double mutants [7]. MEX-3 and GLD-1 are germline-specific RNA-binding proteins that transiently associate with P granules. In *mex-3 gld-1* mutant germlines, P granules persist in germ cells in the distal mitotic zone but are lost from postmitotic cells, which transform into somatic cell types [7]. Here, we examined whether P-granule impairment is sufficient to cause germ cell reprogramming.

Loss of individual P-granule components in *C. elegans* results in sterile adults, but the effects of losing P granules altogether have not been fully assessed. This stems in part from the fact that P granules are comprised of dozens of protein components [11, 12], many of which function redundantly; no single mutation has been demonstrated to abolish P granules. We sought to impair P-granule assembly in *C. elegans* germlines by simultaneously depleting four core P-granule components by RNAi. The core P-granule components PGL-1 and PGL-3 self-associate and nucleate granule formation in the cytoplasm [4, 9, 13], while the core components GLH-1 and GLH-4 help promote the localization of granules to the nuclear periphery [9]. Taking advantage of a new approach developed by Gouda et al. [14], we stitched together RNAi constructs for *pgl-1*, *pgl-3*, *glh-1*, and *glh-4* and inserted this quadruple construct into a vector for feeding RNAi [15]. RNAi feeding was started on worms in the first larval stage. This initial (P0) generation remained fertile (0 of 100 sterile), and 29 of 29 dissected germlines stained positively for PGL-1 (Figure 1A), likely reflecting promotion of germline health by the maternal contribution of P granules and inefficient depletion of PGL-1 in the first generation of RNAi. However, 40 of 100 of their F1 adult progeny were sterile (sterility ranged from 27% to 89% among experiments; see Table S1 available online), with abnormal oocytes and lacking embryos in the uterus (Figure 1B) but still containing sperm as detected by the sperm-specific antibody SP56 (Figure 1C). In fertile F1s, PGL-1 staining was observed in 9 of 22 dissected germlines; PGL-1 staining was not observed (0 of 34) in sterile F1 germlines (Figure 1A; $p = 6.6 \times 10^{-5}$), suggesting that sterility is correlated with more robust depletion of PGL-1 using this RNAi approach. To determine the extent to which P granules are disrupted, we immunostained sterile F1 germlines for DEPS-1, a constitutive P-granule component that promotes GLH-1 expression and PGL-1 localization to P granules [6]. After P-granule RNAi, DEPS-1 did not localize to granules at the nuclear periphery but was instead dispersed throughout the cytoplasm (Figure 1D). Transcript levels of *pgl-1*, *pgl-3*, *glh-1*, and *glh-4* in germlines dissected from F1 worms after RNAi feeding were reduced 24.7-, 13.9-, 2.0-, and 3.7-fold, respectively (Figure 1E). These results show that core P-granule components are either depleted or dispersed in sterile F1 worms after RNAi feeding.

The pan-neuronal marker *unc-119*::GFP has been used in other studies to indicate when germ cells have been reprogrammed to a somatic (neural) fate [7, 8]. This marker was used in conjunction with P-granule RNAi to determine whether compromising P granules causes germ cells to reprogram toward neurons. Expression of this marker was not observed in the germline of control worms but was observed in the germline of some sterile F1 worms following P-granule RNAi. Based on examination of >200 worms per condition and stage, control worms included empty-vector RNAi P0s and F1 generation

*Correspondence: dupdike@mdibl.org

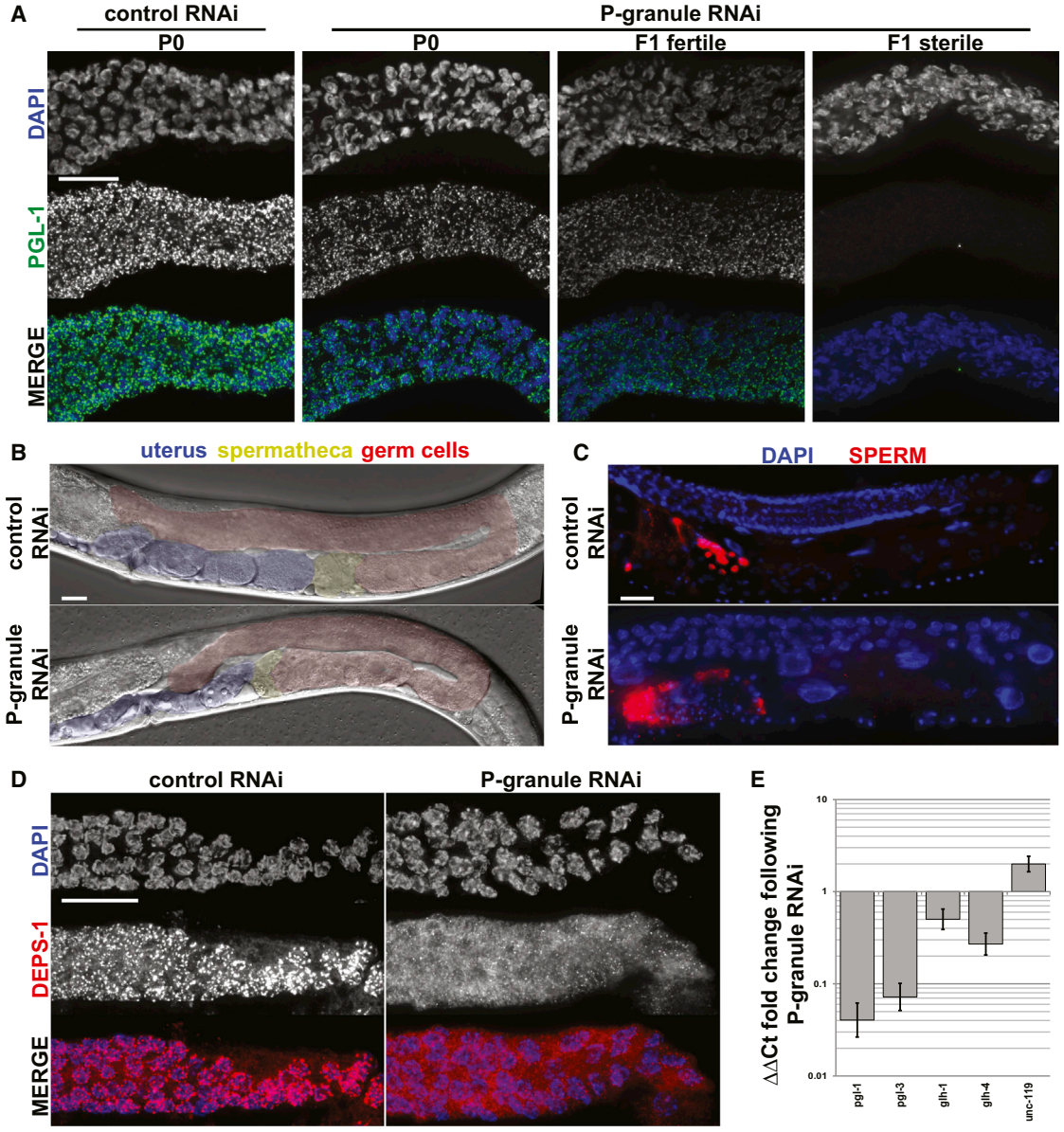

Figure 1. P-Granule RNAi

(A) PGL-1 staining is reduced to below detection in sterile F1 worms following P-granule RNAi. In (A)–(D), scale bars represent 20 μm.
(B) Pseudocolored differential interference contrast image showing germ cell abnormalities in sterile F1 hermaphrodites depleted of P granules.
(C) SP56 staining (red) shows the presence of sperm in P-granule RNAi hermaphrodites.
(D) DEPS-1, a constitutive P-granule component, is no longer perinuclear following P-granule RNAi but is instead dispersed throughout the cytoplasm.
(E) Quantitative PCR comparing changes in transcript levels following P-granule RNAi. Error bars show SD.

worms at the L4 stage and at days 1, 2, and 4 of adulthood; none showed germline GFP expression. Following P-granule RNAi, germline expression of *unc-119*::GFP was not observed in P0s or L4-stage F1s, but was observed in clusters of cells throughout the germline of sterile F1 adults (Figure 2A): 2.4% of day 1 adults ($p = 3.5 \times 10^{-2}$), 10% of day 2 adults ($p = 3.5 \times 10^{-7}$), and 17% of day 4 adults ($p = 4.6 \times 10^{-11}$). All subsequent P-granule RNAi experiments examined F1 worms on the second day of adulthood. F1 males at day 2 of adulthood also showed germline *unc-119*::GFP expression at a similar frequency (5 of 43 = 12%, Figure 2A). P-granule RNAi hermaphrodites displayed a modest 2-fold increase in *unc-119* transcript level in dissected germlines (Figure 1E). To determine whether this 2-fold increase in *unc-119*::GFP transcript level is distributed throughout the germline or is a result of mosaic *unc-119* transcription that correlates with *unc-119*::GFP-expressing cells, we performed single-molecule fluorescence in situ hybridization (FISH) with an *unc-119* probe. FISH signal was not observed in the germline of *unc-119*::GFP worms (0 of 20) but was detected in GFP-positive germlines following P-granule RNAi (7 of 9) (Figure 2B; $p = 2.3 \times 10^{-5}$), suggesting that in wild-type germlines, P granules function to destabilize *unc-119* transcripts or suppress *unc-119* transcription. In germlines that contained *unc-119*::GFP, *unc-119* transcripts were distributed throughout the germline and showed no enrichment in *unc-119*::GFP-expressing germ

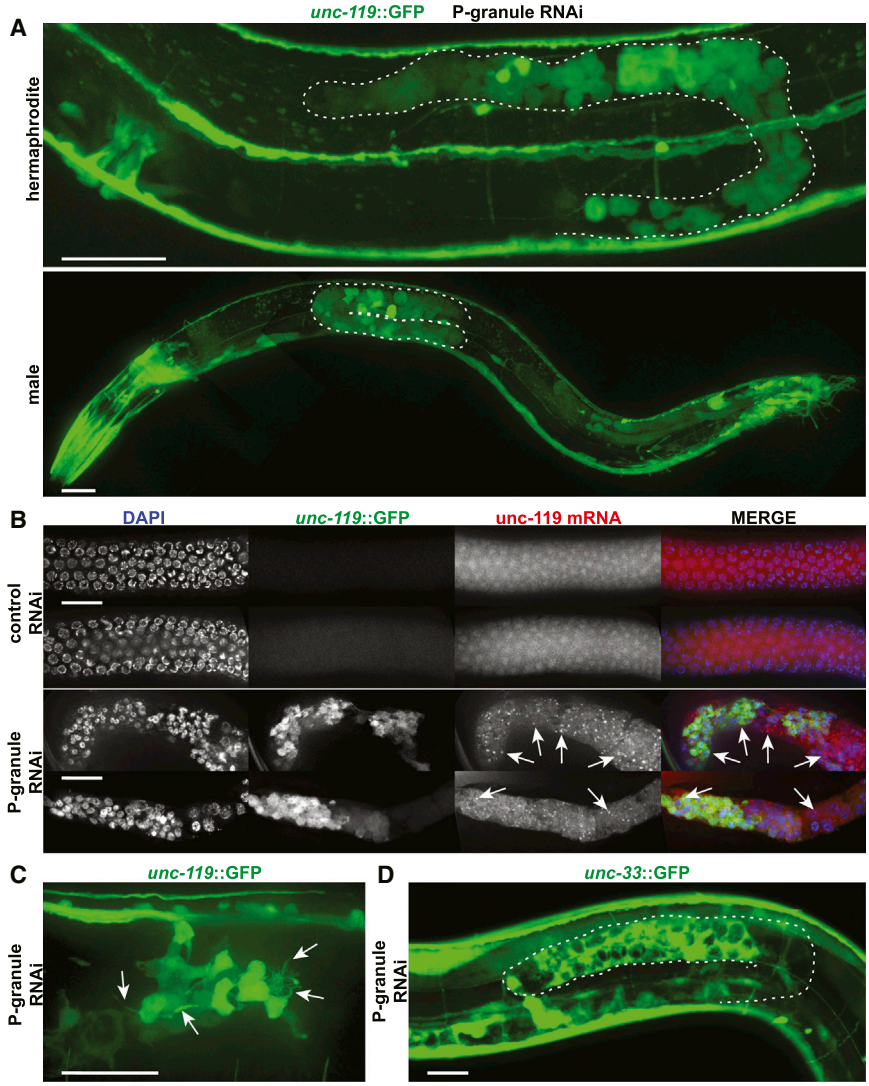

Figure 2. Reprogramming of Germ Cells toward Neurons following P-Granule RNAi

(A) Expression of the pan-neuronal *unc-119*::GFP reporter in the germline (dotted outline) of a hermaphrodite and a male following P-granule depletion.

(B) Single-molecule FISH of *unc-119* mRNA (arrows) and immunostaining of *unc-119*::GFP expression in the germline.

(C) High-resolution image of germ cells expressing *unc-119*::GFP and extending neurite-like projections (arrows).

(D) Expression of the pan-neuronal *unc-33*::GFP reporter in the germline (dotted outline) following P-granule depletion.

In all panels, scale bars represent 20 μm.

cells, suggesting that *unc-119*::GFP expression is also regulated at the level of translation. Often GFP-expressing germ cells had extended neurite-like projections similar to those reported in *mex-3 gld-1* mutant worms [7] (Figure 2C). These results suggest that P granules play a role in maintaining germline totipotency by suppressing expression of somatic factors and somatic differentiation.

The presence of neurite-like projections following P-granule RNAi suggests that germ cells may be differentiating into specific neuronal subtypes. In *C. elegans*, the nervous system comprises 37% of the somatic cells in the adult (302 neurons and 56 glial cells) [16]. In addition to pan-neuronal markers like *unc-119*::GFP, fluorescent neuronal fate reporters exist for virtually all sensory, motor, and interneuron subtypes. To determine whether transformed germ cells represent a specific type of neuron, we performed P-granule RNAi in lines expressing fluorescent reporters of *unc-33* (pan-neuronal); *gcy-5*, *ceh-36*, *lsy-6* (ASE sensory neurons); *cat-1* (dopaminergic and serotonergic neurons); *glr-1* (interneurons); *cnd-1* (ventral cord motor neurons); *unc-47* (GABAergic motor neurons); *pttx-3* (AIY interneurons); *unc-97* (mechanosensory neurons); and reporters expressed in an unspecified subset of neurons (*dhhc-14*, *nhr-52*, *unc-93*, *snb-1*, and *hlh-2*) (Table S1). Of these lines, the only one to show germline expression after P-granule RNAi was the pan-neuronal marker *unc-33*::GFP (Figure 2D; 35 of 103 sterile worms); none of the terminal neuronal reporters were detectably expressed in the germline following P-granule RNAi. Immunostaining of UNC-10, which is detected in axonal extensions, was not visible in *unc-119*::GFP-positive germ cells. Additionally, nuclear granules, which are observed in differentiated neurons, were not seen in *unc-119*::GFP-positive germ cells. Germline expression of the pan-neuronal reporters *unc-119* and *unc-33* and the appearance of neurite-like projections suggest that some germ cells are reprogrammed to neuron-like cells, but there is no evidence to date that these cells are differentiating into a specific neuronal subtype (or subtypes). The absence of reporter gene expression is difficult to interpret, but one possibility is that germ-to-soma reprogramming is being initiated, but not completed, after RNAi depletion of P granules.

To test whether P-granule RNAi enhances the ability of germ cells to differentiate into a specific neuronal subtype in response to a neuronal differentiation signal, we used a transgenic strain in which expression of the neuronal differentiation factor CHE-1 can be induced throughout the body by heat shock [8]. CHE-1 is normally expressed in ASE neurons and activates expression of a number of ASE-specific genes [17, 18]. Heat-shock induction of CHE-1 throughout the worm is not sufficient to reprogram cells into ASE neurons in a wild-type background [8]. However, after depletion of any of several chromatin factors (LIN-53, MES-2, MES-3, MES-6, or MES-4) [8, 19], heat-shock induction of CHE-1 can cause germ cells to differentiate into ASE neurons, as marked by ASE-specific *gcy-5*::GFP expression. Worms carrying *hs*::CHE-1 with either *gcy-5*::GFP or *unc-119*::GFP reporters were fed control or P-granule RNAi. Germline expression of *gcy-5*::GFP was observed in 14% of P-granule RNAi worms following heat shock (42 of 300; $p = 2.3 \times 10^{-10}$) (Figure 3A), but in only 1% of control RNAi worms (3 of 300), showing that depletion of P granules permits CHE-1 expressed in germ cells to drive differentiation of ASE neurons. Germline expression of the

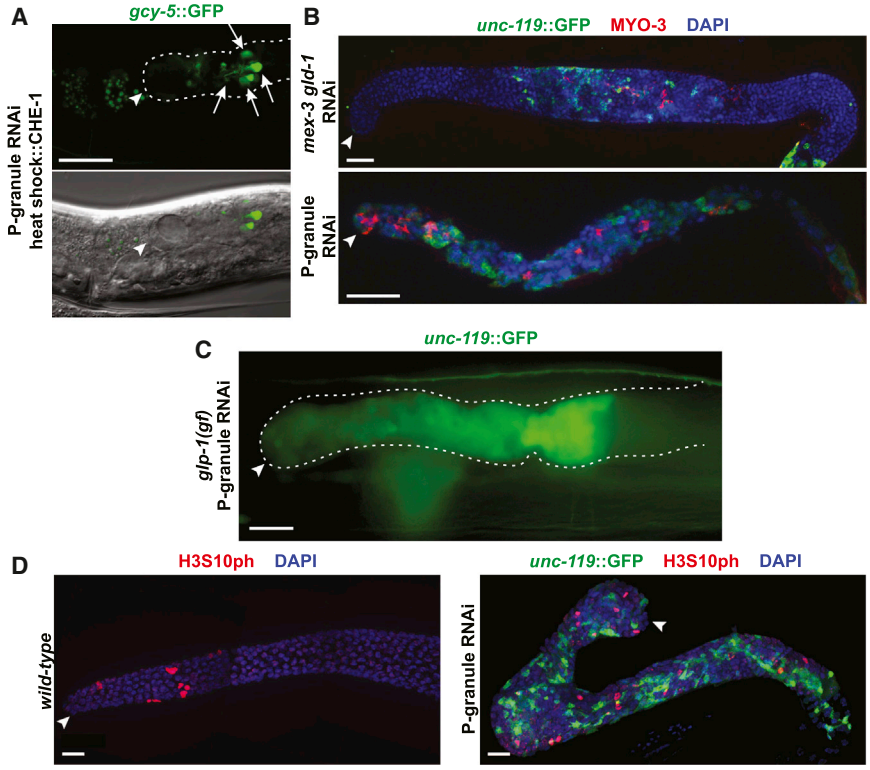

Figure 3. Reprogramming of Mitotic Germ Cells toward Neurons and Muscle

(A) P-granule RNAi permits *gcy-5* expression (arrows) in the germline (dotted outline) after CHE-1 induction.

(B) Muscle (red) and neuronal (green) markers are detected in germlines following either *mex-3 gld-1* RNAi or P-granule RNAi. Both markers are observed in the distalmost region of P-granule RNAi germlines but not of *mex-3 gld-1* RNAi germlines.

(C) The neuronal *unc-119*::GFP marker is observed in the distal germline of P-granule RNAi worms bearing a *glp-1(gf)* mutation that extends the distal mitotic zone.

(D) P-granule RNAi causes germ-toward-soma reprogramming (green) in germlines that contain mitotic cells marked by H3S10ph staining (red).

In all panels, arrowheads mark the distal tip of the germline, and scale bars represent 20 μm.

pan-neuronal *unc-119*::GFP reporter was not observed in control RNAi worms (0 of 100), and expression in P-granule RNAi worms was similar ($p = 8.2 \times 10^{-1}$) with (12 of 100, $p = 3.4 \times 10^{-4}$) and without (10 of 100, $p = 1.5 \times 10^{-3}$) heat shock, suggesting that although expression of CHE-1 in the germline of P-granule RNAi worms drives ASE differentiation, it does not increase the frequency of germline reprogramming. These results show that compromising P granules enhances the ability of germ cells to differentiate into neurons in response to CHE-1, and also suggest that only 10%–14% of worms contain germ cells that are both sufficiently depleted of P granules using this RNAi method and healthy enough to reprogram into neuron-like cells. An attractive possibility is that LIN-53 and the MES proteins provide a chromatin-level barrier to transcribing neuronal genes in the germline and that P granules provide a posttranscriptional barrier to translating stochastically expressed or CHE-1-induced neuronal gene transcripts.

We investigated whether P-granule-depleted germlines express markers of multiple somatic lineages, similar to germline teratomas observed following loss of the translational repressors MEX-3 and GLD-1 [7]. As previously reported by Ciosk et al. [7], we observed expression of both *unc-119*::GFP and a marker of body wall muscle, MYO-3 (detected with the monoclonal 5.6 antibody), in germlines following *mex-3 gld-1* RNAi (Figure 3B; MYO-3 in 20 of 21 *unc-119*::GFP-positive germlines, $p = 2.2 \times 10^{-12}$). We also detected MYO-3 in germlines from sterile worms following P-granule RNAi (4 of 15), but not following control RNAi (0 of 39, $p = 4.3 \times 10^{-3}$). In P-granule RNAi germlines expressing *unc-119*::GFP, 18 of 29 coexpressed MYO-3 ($p = 2.5 \times 10^{-7}$), compared to 0 of 26 following control RNAi. This suggests that germ cells can be reprogrammed toward both neuronal and muscle fates. However, immunostaining of HLH-1, another muscle marker, was not observed in P-granule RNAi germlines. To test for reprogramming toward other somatic lineages, we surveyed a number of fluorescent somatic reporters after P-granule RNAi, including *pha-4* and *grl-11* (pharynx); *myo-2*, *myo-3*, *hlh-1*, *him-4*, and *unc-97* (muscle); *ceh-21* and *egl-27* (all somatic cells); *vab-3* and *hbl-1* (skin, nerve cord, and muscle); and *elt-2* and *end-3* (intestine). Despite seeing consistent expression of *unc-119*::GFP in the germline, we did not observe expression of these other reporters, including the *myo-3* transcriptional reporter, after P-granule RNAi. Somatic-promoter-driven expression of transgenes may not always be a reliable way to test for expression of somatic factors in the germline, especially in cases of partial reprogramming, as gene expression in germ cells is sensitive to chromatin context, 3′ UTRs, and the presence in transgenes of DNA sequences that are not normally expressed in germ cells [20–22]. At present, our findings suggest that P-granule depletion using this RNAi technique causes partial but not complete conversion of germ cells toward neurons and muscle. Finding conditions that promote a higher frequency of conversion would facilitate in-depth analysis of diverse lineage markers, and targeting different combinations of P-granule components may reveal whether it is the incomplete composition or the disassembly of P granules that causes conversion.

P-granule RNAi causes *unc-119*::GFP and MYO-3 expression in clusters of cells throughout the germline, including the distal mitotic zone, which is normally occupied by actively dividing germline stem cells (Figure 3B). This pattern is in contrast to *mex-3 gld-1* mutant and RNAi germlines, in which reprogramming is observed in the zone normally occupied by meiotic germ cells but not in the distal mitotic zone (Figure 3B) [7]. To determine whether reprogramming occurs in proliferating premeiotic germ cells following P-granule RNAi, we introduced the *unc-119*::GFP reporter into a strain bearing a *glp-1* gain-of-function mutation that extends the domain of mitotic germ cells before entry into meiosis [23]. After P-granule RNAi, we observed *unc-119*::GFP expression in the distal extended-mitotic zone of *glp-1(gf)* mutant germlines (Figure 3C; *glp-1(gf)*, 7 of 185; wild-type, 8 of 250). Furthermore, imaging of histone H3 phosphorylated on serine 10 (H3S10ph), a marker of mitosis [24, 25], in P-granule RNAi

germlines expressing *unc-119*::GFP revealed H3S10ph-positive mitotic cells interspersed among *unc-119*::GFP-positive cells (Figure 3D; 11 of 12 dissected germlines). This indicates that reprogramming occurs in germlines undergoing ongoing proliferation but does not rule out the possibility that meiotic germ cells can also be reprogrammed. Our findings distinguish reprogramming after P-granule depletion from reprogramming after loss of MEX-3 and GLD-1: after P-granule depletion, germ cells in the mitotic zone undergo germ-to-ward-soma reprogramming in both hermaphrodites and males, while reprogramming in *mex-3 gld-1* mutants requires entry into meiosis and is not observed in males [7]. Perhaps P granules in the distal germline of *mex-3 gld-1* mutants maintain germline stem cell identity and totipotency, and loss of P granules in the meiotic region of those mutants causes reprogramming.

In *C. elegans*, many RNAs are regulated by germline-expressed RNA-binding proteins that transiently associate with P granules [26–30], and in fact, maximal repression of some germline-expressed transcripts requires P-granule association of those proteins [31–34], but it has not been clear how the P-granule microenvironment participates in this regulation. In the present study, we simultaneously depleted four key P-granule components and severely compromised P-granule integrity, in order to infer the function of P granules in germ cells. Our findings support a model in which P granules selectively degrade or impair the translation of mRNAs that promote neuronal and muscle differentiation. Instead of acting as germ cell determinants, P granules likely act to buffer germ cells from the effects of stochastic mistranscription of genes encoding somatic factors, thereby maintaining germline totipotency and preventing reprogramming of germ cells into somatic cell types. This role may be conserved in mammals, where the germ-granule component Dazl functions in maintenance of pluripotency in mouse primordial germ cells [35]. A critical issue to resolve is how P granules are able to selectively repress somatic, but not germline, factors. One attractive possibility is that selectivity is mediated through small RNAs and P-granule-associated Argonautes, which enable germ cells to distinguish between "self" and "nonself" transcripts (reviewed in [36]). Future experiments will test the contributions of individual P-granule proteins and protein families (e.g., PGL-1 and the PGL family) to preventing germ-toward-soma reprogramming, identify RNAs associated with P granules and the factors that contribute to that association, and elucidate how P granules regulate the turnover and translation of their associated RNAs.

Supplemental Information

Supplemental Information includes one table and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.03.015.

Acknowledgments

We are grateful to those who provided reagents for this study, including Oliver Hobert, Baris Tursun, Michael Krauss, Mike Nonet, Ian Hope, Peter Roy, Bob Horvitz, Donald Moerman, Hiroshi Kimura, the Developmental Studies Hybridoma Bank (developed under NICHD and maintained by the University of Iowa), Bar Harbor Biotechnology, and the Caenorhabditis Genetics Center (funded by NIH grant P40 OD010440). This research was supported by NIH grant P20GM104318 to D.L.U., California Institute for Regenerative Medicine Scholar Training Grant TG2-01157 to D.L.U., an NHGRI-supported Research Mentoring Institute fellowship to A.K.K., and NIH grant GM34059 and Ellison Senior Scholar in Aging Award AG-SS-3098-12 to S.S.

Received: October 18, 2013
Revised: February 5, 2014
Accepted: March 4, 2014
Published: April 17, 2014

References

1. Gao, M., and Arkov, A.L. (2013). Next generation organelles: structure and role of germ granules in the germline. Mol. Reprod. Dev. *80*, 610–623.
2. Santos, A.C., and Lehmann, R. (2004). Germ cell specification and migration in Drosophila and beyond. Curr. Biol. *14*, R578–R589.
3. Gallo, C.M., Wang, J.T., Motegi, F., and Seydoux, G. (2010). Cytoplasmic partitioning of P granule components is not required to specify the germline in C. elegans. Science *330*, 1685–1689.
4. Kawasaki, I., Amiri, A., Fan, Y., Meyer, N., Dunkelbarger, S., Motohashi, T., Karashima, T., Bossinger, O., and Strome, S. (2004). The PGL family proteins associate with germ granules and function redundantly in Caenorhabditis elegans germline development. Genetics *167*, 645–661.
5. Spike, C.A., Meyer, N., Racen, E., Orsborn, A., Kirchner, J., Kuznicki, K., Yee, C., Bennett, K., and Strome, S. (2008). Genetic analysis of the Caenorhabditis elegans GLH family of P-granule proteins. Genetics *178*, 1973–1987.
6. Spike, C.A., Bader, J., Reinke, V., and Strome, S. (2008). DEPS-1 promotes P-granule assembly and RNA interference in C. elegans germ cells. Development *135*, 983–993.
7. Ciosk, R., DePalma, M., and Priess, J.R. (2006). Translational regulators maintain totipotency in the *Caenorhabditis elegans* germline. Science *311*, 851–853.
8. Tursun, B., Patel, T., Kratsios, P., and Hobert, O. (2011). Direct conversion of C. elegans germ cells into specific neuron types. Science *331*, 304–308.
9. Updike, D.L., Hachey, S.J., Kreher, J., and Strome, S. (2011). P granules extend the nuclear pore complex environment in the C. elegans germ line. J. Cell Biol. *192*, 939–948.
10. Nüsslein-Volhard, C., Frohnhöfer, H.G., and Lehmann, R. (1987). Determination of anteroposterior polarity in Drosophila. Science *238*, 1675–1681.
11. Updike, D.L., and Strome, S. (2010). P granule assembly and function in Caenorhabditis elegans germ cells. J. Androl. *31*, 53–60.
12. Tohsato, Y., Monobe, K., Suzuki, K., Hayano, T., Kawasaki, I., and Ito, M. (2012). Comparative proteomic analysis reveals differentially expressed proteins in Caenorhabditis elegans pgl-1 mutants grown at 20°C and 25°C. J. Proteomics *75*, 4792–4801.
13. Hanazawa, M., Yonetani, M., and Sugimoto, A. (2011). PGL proteins self associate and bind RNPs to mediate germ granule assembly in C. elegans. J. Cell Biol. *192*, 929–937.
14. Gouda, K., Matsunaga, Y., Iwasaki, T., and Kawano, T. (2010). An altered method of feeding RNAi that knocks down multiple genes simultaneously in the nematode Caenorhabditis elegans. Biosci. Biotechnol. Biochem. *74*, 2361–2365.
15. Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M., et al. (2003). Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. Nature *421*, 231–237.
16. Hobert, O. (2010). Neurogenesis in the nematode Caenorhabditis elegans. WormBook, 1–17.
17. Etchberger, J.F., Lorch, A., Sleumer, M.C., Zapf, R., Jones, S.J., Marra, M.A., Holt, R.A., Moerman, D.G., and Hobert, O. (2007). The molecular signature and cis-regulatory architecture of a C. elegans gustatory neuron. Genes Dev. *21*, 1653–1674.
18. Uchida, O., Nakano, H., Koga, M., and Ohshima, Y. (2003). The C. elegans che-1 gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons. Development *130*, 1215–1224.
19. Patel, T., Tursun, B., Rahe, D.P., and Hobert, O. (2012). Removal of Polycomb Repressive Complex 2 makes C. elegans germ cells susceptible to direct conversion into specific somatic cell types. Cell Rep. *2*, 1178–1186.
20. Merritt, C., Rasoloson, D., Ko, D., and Seydoux, G. (2008). 3′ UTRs are the primary regulators of gene expression in the C. elegans germline. Curr. Biol. *18*, 1476–1482.

21. Kelly, W.G., Xu, S., Montgomery, M.K., and Fire, A. (1997). Distinct requirements for somatic and germline expression of a generally expressed Caenorhabditis elegans gene. Genetics *146*, 227–238.
22. Shirayama, M., Seth, M., Lee, H.-C., Gu, W., Ishidate, T., Conte, D., Jr., and Mello, C.C. (2012). piRNAs initiate an epigenetic memory of nonself RNA in the C. elegans germline. Cell *150*, 65–77.
23. Pepper, A.S.-R., Killian, D.J., and Hubbard, E.J.A. (2003). Genetic analysis of Caenorhabditis elegans glp-1 mutants suggests receptor interaction or competition. Genetics *163*, 115–132.
24. Crittenden, S.L., Bernstein, D.S., Bachorik, J.L., Thompson, B.E., Gallegos, M., Petcherski, A.G., Moulder, G., Barstead, R., Wickens, M., and Kimble, J. (2002). A conserved RNA-binding protein controls germline stem cells in Caenorhabditis elegans. Nature *417*, 660–663.
25. Stevens, D., Oegema, K., and Desai, A. (2013). Meiotic double-strand breaks uncover and protect against mitotic errors in the C. elegans germline. Curr. Biol. *23*, 2400–2406.
26. Schisa, J.A. (2012). New insights into the regulation of RNP granule assembly in oocytes. Int. Rev. Cell Mol. Biol. *295*, 233–289.
27. Boag, P.R., Atalay, A., Robida, S., Reinke, V., and Blackwell, T.K. (2008). Protection of specific maternal messenger RNAs by the P body protein CGH-1 (Dhh1/RCK) during Caenorhabditis elegans oogenesis. J. Cell Biol. *182*, 543–557.
28. Noble, S.L., Allen, B.L., Goh, L.K., Nordick, K., and Evans, T.C. (2008). Maternal mRNAs are regulated by diverse P body-related mRNP granules during early Caenorhabditis elegans development. J. Cell Biol. *182*, 559–572.
29. Sengupta, M.S., Low, W.Y., Patterson, J.R., Kim, H.-M., Traven, A., Beilharz, T.H., Colaiácovo, M.P., Schisa, J.A., and Boag, P.R. (2013). ifet-1 is a broad-scale translational repressor required for normal P granule formation in C. elegans. J. Cell Sci. *126*, 850–859.
30. Voronina, E., Seydoux, G., Sassone-Corsi, P., and Nagamori, I. (2011). RNA granules in germ cells. Cold Spring Harb. Perspect. Biol. *3*, a002774.
31. Gallo, C.M., Munro, E., Rasoloson, D., Merritt, C., and Seydoux, G. (2008). Processing bodies and germ granules are distinct RNA granules that interact in C. elegans embryos. Dev. Biol. *323*, 76–87.
32. Subramaniam, K., and Seydoux, G. (1999). nos-1 and nos-2, two genes related to Drosophila nanos, regulate primordial germ cell development and survival in Caenorhabditis elegans. Development *126*, 4861–4871.
33. Voronina, E., and Seydoux, G. (2010). The C. elegans homolog of nucleoporin Nup98 is required for the integrity and function of germline P granules. Development *137*, 1441–1450.
34. Voronina, E., Paix, A., and Seydoux, G. (2012). The P granule component PGL-1 promotes the localization and silencing activity of the PUF protein FBF-2 in germline stem cells. Development *139*, 3732–3740.
35. Haston, K.M., Tung, J.Y., and Reijo Pera, R.A. (2009). Dazl functions in maintenance of pluripotency and genetic and epigenetic programs of differentiation in mouse primordial germ cells in vivo and in vitro. PLoS One *4*, e5654.
36. Kasper, D.M., Gardner, K.E., and Reinke, V. (2013). Homeland security in the C. elegans germ line: insights into the biogenesis and function of piRNAs. Epigenetics *9*, 1–13.